

## Supplementary Information: Supplementary Figures

### Patient-derived xenograft culture-transplant system for investigation of human breast cancer metastasis

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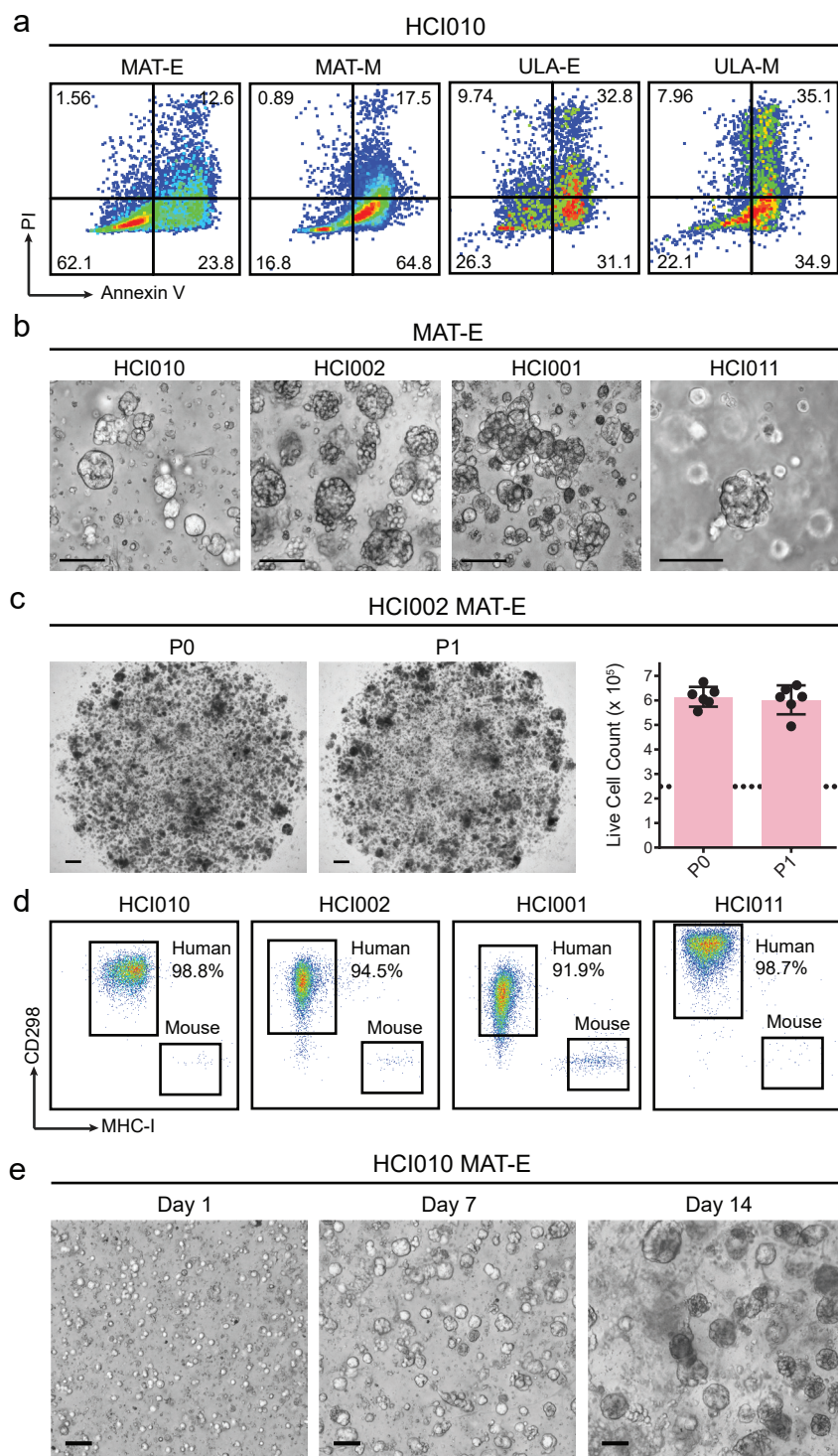
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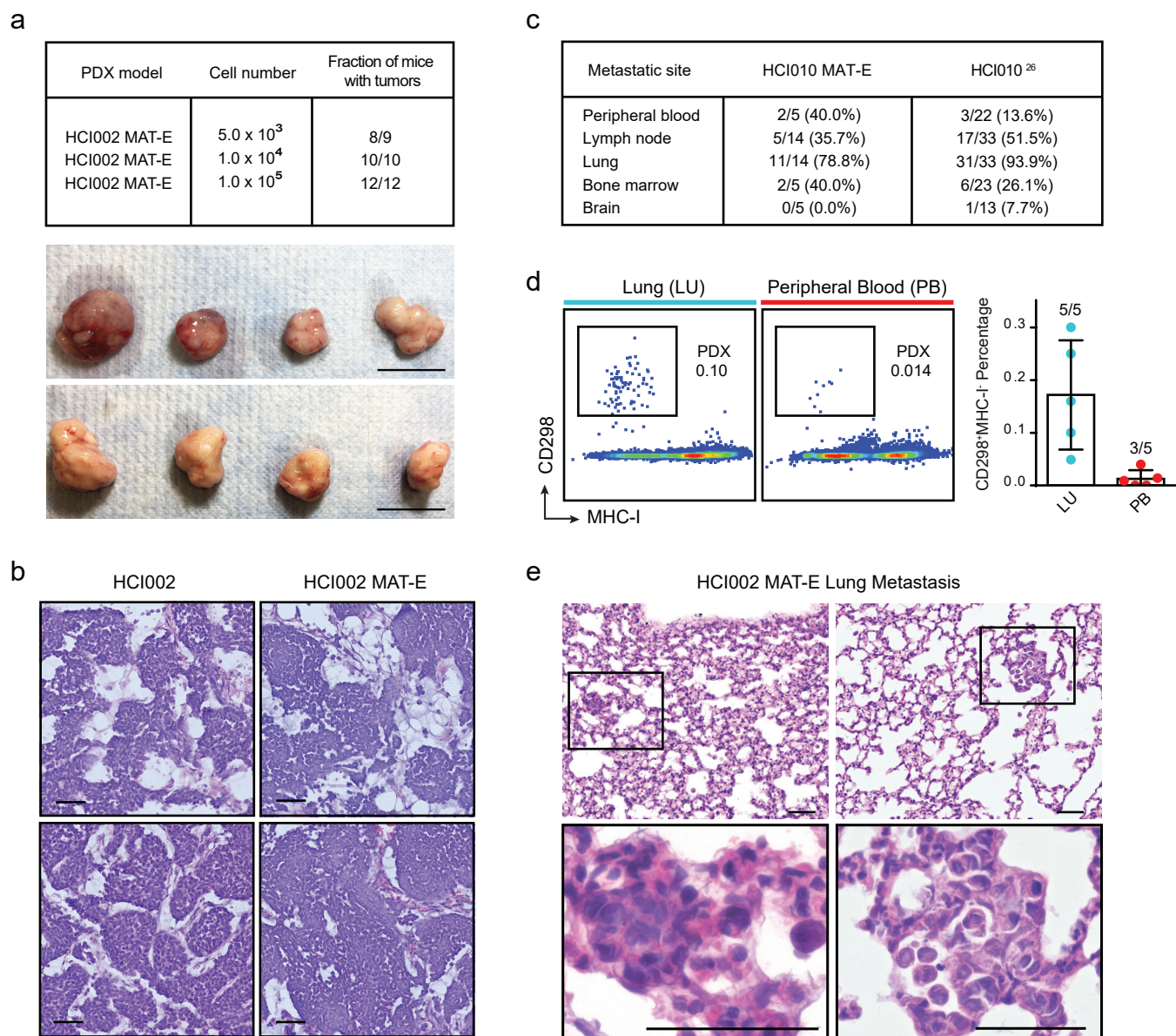
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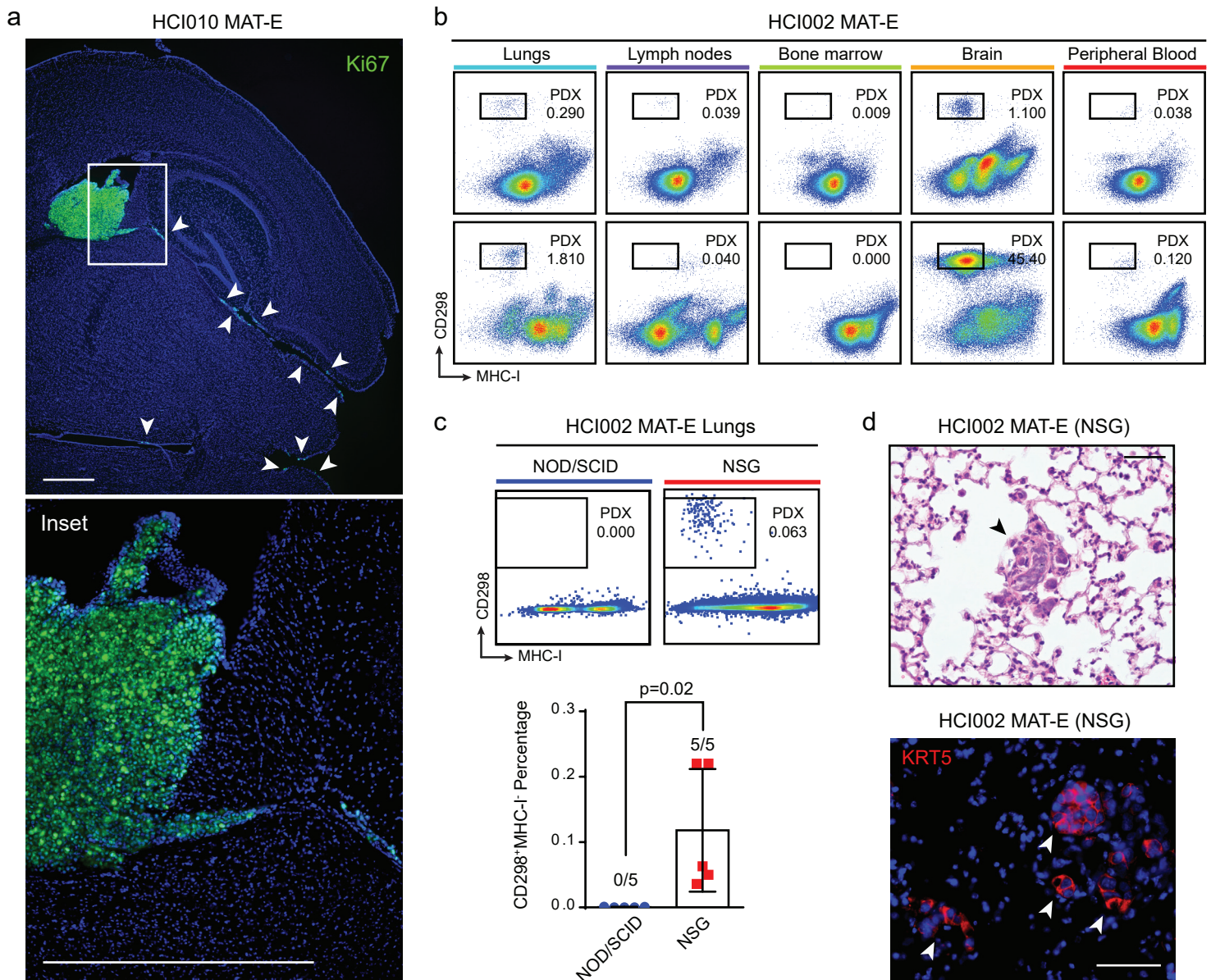


**Supplementary Figure 1: Comparison of PDX cell culture conditions.** (a) Representative flow cytometry plots show viability of HCl010 cells by aV and PI staining after culture as described in Fig 1a. (b) Representative brightfield images show spheres generated by HCl010, HCl002, HCl001 and HCl011 tumor cells grown in MAT-E culture conditions. Scale bar= 100  $\mu$ m. (c) Analysis of cell number and viability in HCl002 passaging experiments. P0 and P1 cells were plated at  $2.5 \times 10^5$  cells/well (dashed line) in MAT-E conditions and quantified nine days later by trypan blue exclusion. Brightfield images (left panels) show representative wells containing spheroid structures at day nine. Scale bars = 400  $\mu$ m. Bar graph (right panel) shows total viable HCl002 cell number counted at day nine in P0 and P1 (n=6 wells each). Data represented as mean  $\pm$  s.d. (d) Flow cytometry analysis to determine species identity of spheres generated in (b). Representative plots show the percent of human CD298<sup>+</sup>MHC-I<sup>+</sup> tumor cells in each culture. (e) Time-lapse imaging of sphere growth from HCl010 cells in MAT-E conditions. Representative brightfield images at day 1, 7, and 14 are shown. See also Supplementary videos 1-3. Scale bar= 100  $\mu$ m.



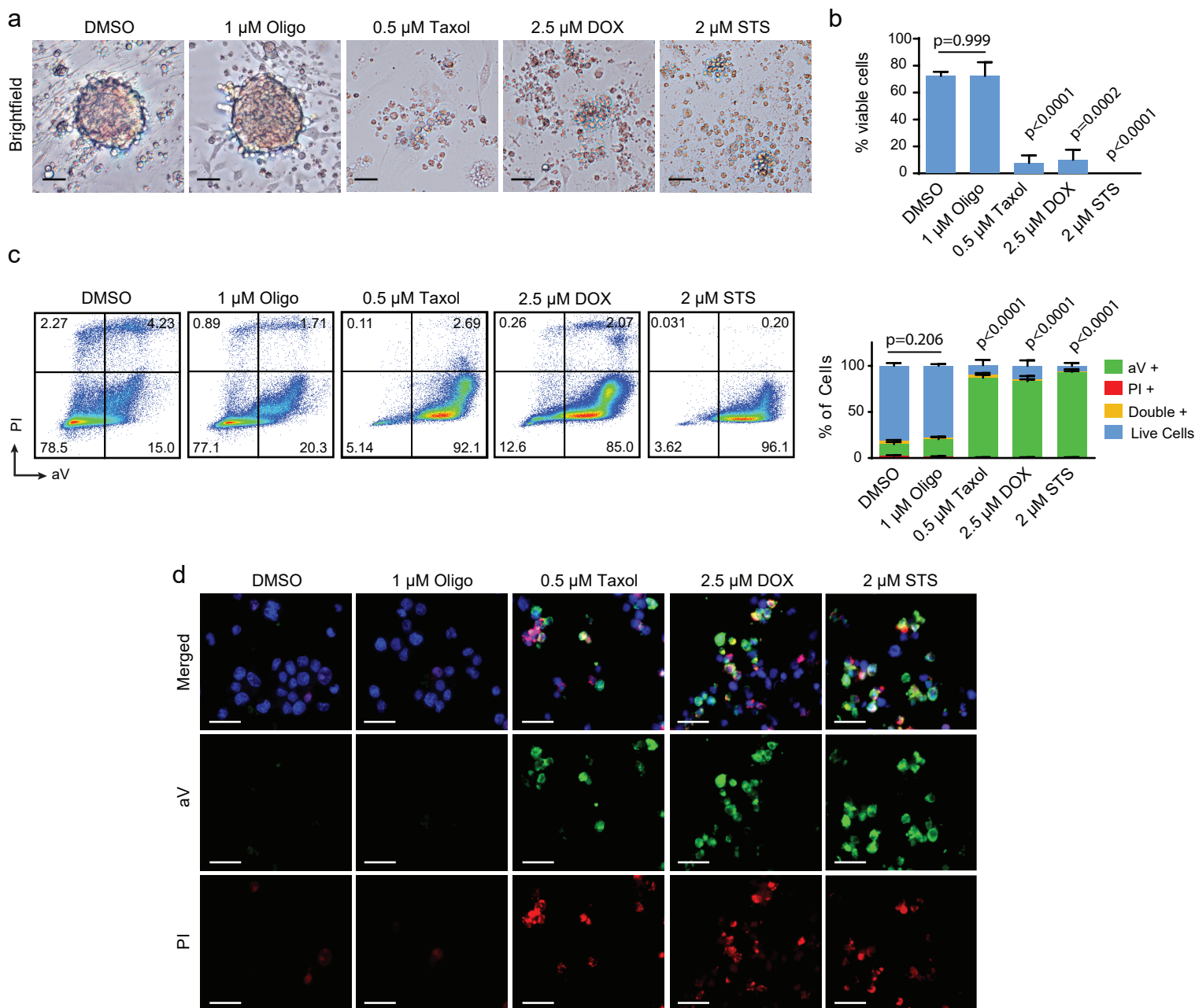
**Supplementary Figure 2: Analysis of primary tumors and spontaneous metastases generated by cultured PDX cells.** **(a)** Serial dilution and transplantation analysis to determine tumorigenic capacity of cultured cells. HCI002 cells were cultured in MAT-E conditions and injected orthotopically into NOD/SCID mice at increasing dilution ( $5.0 \times 10^3 - 1.0 \times 10^5$ ). Table (top) shows the fraction of tumors generated at each dilution. Representative images (bottom) show primary tumors generated from orthotopic transplantation of  $1 \times 10^5$  cells after 12 weeks *in vivo*. Scale bar = 1cm. **(b)** Histopathological analysis of tumors generated from uncultured (HCI002) and cultured (HCI002 MAT-E) cells. Representative images show tumor sections stained with hematoxylin and eosin (H&E). Scale bar = 50  $\mu$ m. **(c)** Table shows the percentage of mice positive for metastasis in indicated tissues following orthotopic transplantation of cultured (HCI010 MAT-E) vs uncultured (HCI010) cells. Data for uncultured cells is taken from Lawson et al., 2015.<sup>26</sup> **(d)** Quantification of spontaneous metastasis in animals transplanted with  $1 \times 10^5$  cultured HCI002 cells. Representative plots (left) show CD298<sup>+</sup>MHC-I<sup>-</sup> human metastatic PDX cells in the lung and peripheral blood by flow cytometry. Bar graph (right) shows quantification of percentage of metastatic cells in a cohort of transplanted animals (n=5). Fractions indicate the number of tissues with metastasis, defined by >0.005% CD298<sup>+</sup>MHC-I<sup>-</sup> cells. Data is represented as the mean  $\pm$  s.d. LU = Lung, PB = peripheral blood. **(e)** Representative images of metastatic lesions in the lungs of animals transplanted with cultured HCI002 cells identified by H&E staining and histopathological analysis. Scale bar = 50  $\mu$ m.



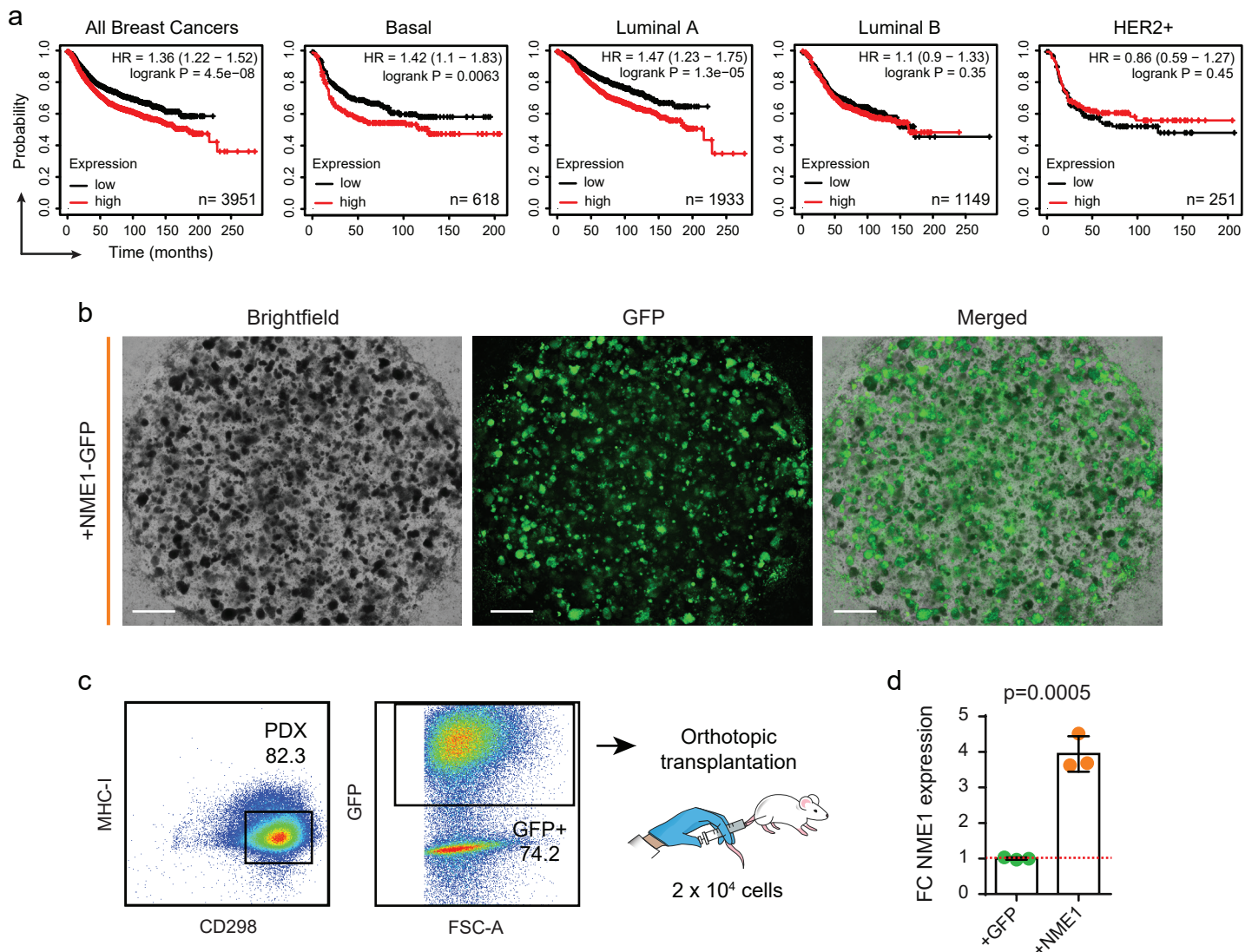


**Supplementary Figure 3: Characterization of experimental metastasis produced by PDX sphere cells. (a)** IF staining for Ki67 in brain metastatic lesion from Fig 5d. Inset shows higher power visualization of Ki67<sup>+</sup> nuclei in 40  $\mu$ m brain section. Arrows indicate micrometastatic lesions. Scale bar = 800  $\mu$ m. **(b)** Analysis of metastatic spread following i.c. injection of cultured HCl002 cells *in vivo*. Representative flow cytometry plots show CD298<sup>+</sup>MHC-I<sup>+</sup> human metastatic cells in the lungs, lymph nodes, bone marrow, brain, and blood 8 weeks following i.c. injection of  $5 \times 10^5$  cultured HCl002 cells into NOD/SCID mice. **(c)** Comparison of experimental metastasis from cultured HCl002 cells in NOD/SCID and NSG mice. Flow cytometry plots show percent of human CD298<sup>+</sup>MHC-I<sup>+</sup> metastatic cells in the lungs of representative mice 8 weeks following i.v. injection of  $5 \times 10^5$  HCl002 cultured cells (top plots). Bar graph shows quantification of percentage of metastatic cells in a cohort of transplanted animals (n=5) (bottom). Fractions indicate the number of lungs with metastasis, as defined by  $>0.005\%$  CD298<sup>+</sup>MHC-I<sup>+</sup> cells. Data is represented as the mean  $\pm$  s.d.. *P*-value was determined by unpaired t-test. **(d)** Images show representative metastatic lesions (arrows) in the lungs of NSG mice identified by H&E staining (top) and IF staining for KRT5 (bottom). Scale bar = 50  $\mu$ m.





**Supplementary Figure 4: Cytotoxicity and cell death analysis of PDX cells post drug treatment.** (a) Z-stack brightfield images of representative HCl002 MAT-E spheroids grown for two weeks and treated with the indicated drugs for 72 hours. Scale bar= 50  $\mu$ m. Oligo= oligomycin; DOX= doxorubicin; STS= staurosporine. (b) Trypan blue exclusion viability assay of HCl002 MAT-E cells grown for two weeks and treated with the indicated drugs for 72 hours. Values expressed as mean  $\pm$  s.d. from triplicate wells (n=3). *P*-values were determined by unpaired t-tests of each condition vs. the DMSO control group. Taxol, DOX and STS were used as positive controls for cell death. (c) Representative flow cytometry plots of aV and PI dead cell analysis of HCl002 MAT-E cells treated for 72 hours. Bar graph shows percent of live and dead HCl002 cells by aV and PI. Data represented as mean  $\pm$  s.d. (n=3). *P*-values were determined by unpaired t-tests of each condition vs. the DMSO control group for the percent of aV<sup>+</sup>PI<sup>-</sup> viable cells. Taxol, DOX and STS were used as positive controls for cell death. (d) Representative aV and PI fluorescence micrographs of HCl002 MAT-E cells treated for 72 hours. Blue= Hoechst nuclear counterstain. Scale bar= 25  $\mu$ m.



**Supplementary Figure 5: NME1 overexpression in cultured PDX cells and its relevance in patient breast cancer prognosis.** (a) Kaplan-Meier (KM) curves show relapse free survival (RFS) in breast cancer patients by tumor subtype using the KM plotter database<sup>56</sup>, based on their primary tumor expression of NME1. *P*-values were determined via a log-rank test. (b) Images show GFP expression in PDX spheres cultures following lentiviral transduction *in vitro*. HCl010 sphere cells were transduced with +NME1-GFP lentivirus at MOI=25 and re-seeded in MAT-E culture at a density of  $2 \times 10^5$  cells per well. Images show z-stack micrographs of individual wells containing P1 spheres 3 weeks later. Scale bar= 800  $\mu$ m. (c) Flow cytometry plots show gating strategy for sorting transduced human GFP<sup>+</sup>CD298<sup>+</sup>MHC-I<sup>-</sup> PDX cells for orthotopic transplantation and qPCR analysis. (d) Bar graph shows qPCR quantification of NME1 expression in control (+GFP) and NME1-GFP (+NME1) transduced cells. Values are plotted as fold change (FC) in NME1 expression in +NME1 relative to +GFP cells and represented as mean  $\pm$  s.d. *P*-value determined by unpaired t-test.